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Review

Making an Effort to Listen: Mechanical Amplification in the Ear

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The inner ear's performance is greatly enhanced by an active process defined by four features: amplification, frequency selectivity, compressive nonlinearity, and spontaneous otoacoustic emission. These characteristics emerge naturally if the mechanoelectrical transduction process operates near a dynamical instability, the Hopf bifurcation, whose mathematical properties account for specific aspects of our hearing. The active process of nonmammalian tetrapods depends upon active hair-bundle motility, which emerges from the interaction of negative hair-bundle stiffness and myosin-based adaptation motors. Taken together, these phenomena explain the four characteristics of the ear's active process. In the high-frequency region of the mammalian cochlea, the active process is dominated instead by the phenomenon of electromotility, in which the cell bodies of outer hair cells extend and contract as the protein prestin alters its membrane surface area in response to changes in membrane potential.

Introduction

The receptor cells of most sensory organs must amplify their signals in order to separate them from background noise. Photoreceptors, for example, use a biochemical cascade to enhance their responses several thousand-fold after transduction has been accomplished. Uniquely among vertebrate sensory receptors, hair cells instead use a mechanical active process to amplify their *inputs*. When sound reaches the cochlea, it elicits mechanical vibrations that are distributed to hair cells and transduced into an electrical response by their mechanoreceptive hair bundles. At the same time, however, the hair cells perform work by increasing the magnitude of their mechanical input. This amplification of the stimulus constitutes positive feedback that enhances the sensitivity of hearing by countering the loss of energy through the viscous dissipation that accompanies the motion of hair bundles and other structures through the liquids of the inner ear (Gold, 1948). Amplification occurs not only in the cochlea, but also in other organs of the acousticolateralis sensory system, and may prove to be a general feature of hair cells (cf. Hudspeth et al., 2000). The auditory receptors of some invertebrates also employ amplification, though its basis has been explored less extensively.

The ear's amplifier is generally called the "active process," a term both of whose components should be qualified. The essence of activity is power gain: "active" amplification occurs only if the energy output of a system exceeds its input. When this is so, the principle of conservation of energy implies that the amplifier—in the present context, the hair cell—has contributed the balance of the energy. The second qualification is that there may be more than one "process" by which amplification occurs in the ear. Although the term "active process" is employed here to encompass all the phenomena associated with amplification, the reader should bear in mind that power amplification may proceed by different avenues in different receptor organs or even in the hair cells of a single organ.

The hair cells of all vertebrates have a similar structure and transduce mechanical stimuli in the same way. Each of these epithelial cells is surmounted by a hair bundle, an erect cluster of 20–300 cylindrical processes called stereocilia (Figures 1A–1C). Because the stereocilia grow systematically in length along the hair bundle, the top surface of the bundle is beveled like the tip of a hypodermic needle. At least during the ear's development, a lone axonemal cilium stands at the hair bundle's tall edge. When a sound reaches the ear, the mechanical energy in this stimulus deflects hair bundles, each of whose constituent stereocilia bends at its base. This deflection causes a shearing motion between contiguous stereocilia that is detected by mechanosensitive ion channels situated near the stereociliary tips (Figure 1D). These transduction channels, whose molecular identity remains unknown, are thought to be gated by the tension in the cadherin-based tip links that couple adjacent stereocilia. This tension is additionally determined by molecular motors containing myosin-1c molecules. As the motors scuttle up and down the stereocilia, adjusting the tension in the tip links, a hair cell adapts to sustained deflection of its hair bundle.

The Active Process of the Inner Ear

Throughout the tetrapod vertebrates, four phenomena characterize the ear's active process (cf. Manley 2000, 2001). The most important is amplification (Figure 2A). In the ears of many species, including humans, the threshold of normal hearing lies at sound-pressure levels around zero decibels (0 dB). This level of sensitivity implies that we can hear stimuli down to a limit imposed by thermal vibrations in the ear. Within minutes after a cochlea has been deprived of energy, however, the threshold of auditory responsiveness rises by 40–60 dB: in other words, the ear's sensitivity falls to less than 1% of its normal value (Ruggero and Rich, 1991). This extraordinary change demonstrates the active process's profound capacity for amplification.

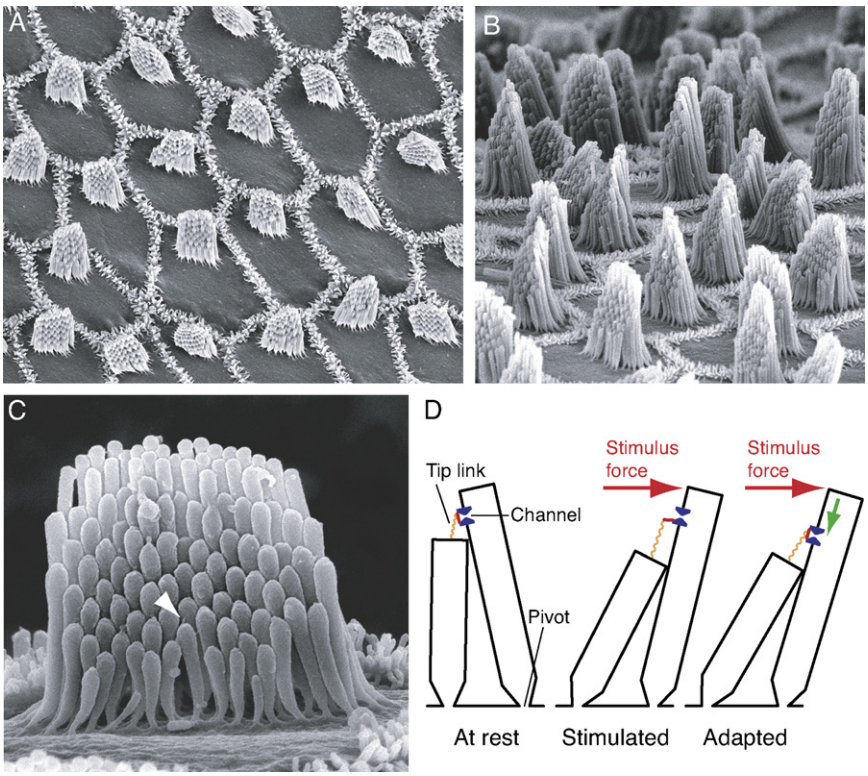

Figure 1. Hair Cells and Their Transduction Process

(A) The sensory epithelium of the chicken cochlea displays a regular, hexagonal array of short hair cells bordered by narrow, microvillus-bearing supporting cells. These short hair cells, which receive no afferent innervation but copious efferent innervation, are thought to contribute to transduction through active hair-bundle motility.

(B) A lateral view of hair bundles from the same preparation emphasizes the systematic increase in stereociliary length across each hair bundle. Deflecting the top of any of these bundles to the right would depolarize the associated hair cell; leftward motion would produce a hyperpolarization.

(C) A higher-power view of a single hair bundle shows the orderly array of stereocilia, some of which remain connected by tip links (arrowhead).

(D) This schematic depiction of a resting hair bundle shows two stereocilia connected by a tip link attached to a transduction channel (left diagram). Deflection of the bundle by a positively directed mechanical stimulus bends the stereociliary pivots, tenses the tip link, and opens the transduction channel, allowing K^+ and Ca^{2+} to enter the cytoplasm and depolarize the hair cell (middle diagram). Ca^{2+} that enters through the channel then interacts with a molecular motor comprising myosin-1c molecules and causes it to slip down the stereocilium's actin cytoskeleton. The reduced tension in the tip link permits the channel to reclose in the process of adaptation (right diagram).

Our ability to recognize the sources of acoustic stimuli depends upon the cochlea's capacity to decompose sounds into their constituent frequencies. A second feature of the active process, which enhances this tuning ability, is sharpened frequency selectivity (Figure 2B). As one moves along the cochlea, adjacent cells respond best to successive pitches, so that the cochlea bears a tonotopic map. In mammals, birds, and many reptiles, low frequencies are represented at the apex of the cochlea and high pitches at the base; lizards display more varied and complex tonotopic patterning. The sharpness of tuning in a normal cochlea reflects the narrow range of frequencies that excite a given hair cell, especially for stimulation near threshold. When the active process is disrupted, however, the ear's decreased sensitivity is accompanied by a severe degradation in frequency selectivity (Ruggero and Rich, 1991). This finding implies that the active process is highly tuned.

Compressive nonlinearity is the third characteristic of the active process (Figure 2C). In the mammalian auditory system, a threshold stimulus of 0 dB evokes a basilar-membrane oscillation near ±0.1 nm. The loudest tolerable sound—a jet plane encountered on the tarmac, Mötley Crüe heard from the pit—measures 120 dB but moves the basilar membrane by only ±10 nm. An input a million times the threshold amplitude, in other words, yields an oscillation only a hundred times the threshold response, indicating that the growth of the output is greatly compressed relative to that of the input. Accordingly, the basilar membrane's sensitivity is characterized, not by a linear relation, but by a power law: the output scales as the one-third power of the input (Ruggero et al., 1997).

The fourth and especially striking manifestation of the active process is spontaneous otoacoustic emission (Figure 2D). In a quiet environment, the ears of many species from all tetrapod classes can emanate sound continuously at one or more frequencies (cf. Probst, 1990; Manley and Köppl, 1998). Because this spontaneous otoacoustic emission can sometimes be detected at a distance of several wavelengths from the ear, the phenomenon unequivocally involves the generation and radiation of acoustic energy. Additional forms of otoacoustic emission exist. When stimulated simultaneously with two or more tones, for example, the cochlea produces sounds of still other frequencies, so-called distortion-product otoacoustic emissions. Passing current through the cochlea also elicits sound by the process of electrically evoked otoacoustic emission. Although these signals are almost certainly associated with the active process, the fact that an acoustical or electrical stimulus is required in their generation implies that the energy source cannot be assigned entirely to the cochlea.

The Hopf Bifurcation

Although the four characteristics of the active process were discovered independently, their joint occurrence in many species provides a potent argument for a common underlying mechanism. Remarkably enough, as was recognized a decade ago, the four features emerge together when a dynamical system operates near a particular type of instability called the Hopf bifurcation (Choe et al., 1998; Camalet et al., 2000; Eguíluz et al., 2000).

Dynamical systems analysis permits the identification of generic behaviors in the temporal evolution of any system, whether

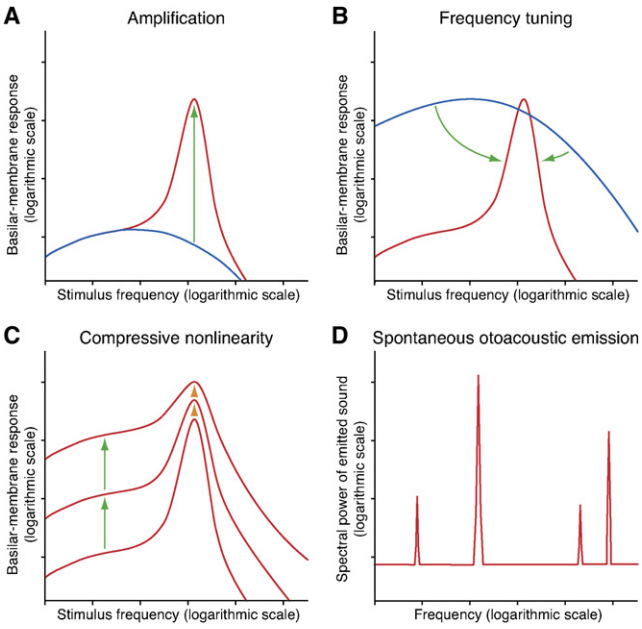

Figure 2. Characteristics of the Ear's Active Process

(A) An input-output relation for the mammalian cochlea relates the magnitude of vibration at a specific position along the basilar membrane to the frequency of stimulation at a particular intensity. Amplification by the active process renders the actual cochlear response (red) over 100-fold as great as the passive response (blue). Note the logarithmic scales in this and the subsequent panels.

(B) As a result of the active process, the observed basilar-membrane response (red) is far more sharply tuned to a specific frequency of stimulation, the natural frequency, than is a passive response driven to the same peak magnitude by much stronger stimulation (blue).

(C) Each time the amplitude of stimulation is increased 10-fold, the passive response distant from the natural frequency grows by an identical amount (green arrows). For the natural frequency at which the active process dominates, however, the maximal response of the basilar membrane increases by only $\sqrt[3]{10}$, a factor of about 2.15 (orange arrowheads). This compressive nonlinearity implies that the basilar membrane is far more sensitive than a passive system at low stimulus levels, but approaches the passive level of responsiveness as the active process saturates for loud sounds.

(D) The fourth characteristic of the active process is spontaneous otoacoustic emission, the unprovoked production of one or more pure tones by the ear in a very quiet environment. For humans and many other species, the emitted sounds differ between individuals and from ear to ear but are stable over months or even years.

a mechanical oscillator, a set of coupled chemical reactions, or a beating heart (cf. Strogatz, 1994). In such an analysis, a bifurcation is said to occur when a small alteration in the value of one parameter, the so-called control parameter, causes a *qualitative* change in the system's behavior. At the critical point of a Hopf bifurcation, a tiny change in the control parameter shifts a system between two regimes (Figure 3). On one side of the bifurcation, the system can actively amplify and tune its inputs. At the same time, though, the system remains stable in that its response relaxes to zero after the input is discontinued. This behavior typifies, for example, a public-address system adjusted to a moderate gain. On the other side of the Hopf bifurcation, the system is unstable: it oscillates spontaneously even in the absence of an input. A similar circumstance pertains for the public-address system when its gain becomes so great that it produces a continuous howling noise.

Mathematical analysis indicates that a system operating on the stable side of a Hopf bifurcation exhibits the first three characteristics of the ear's active process: amplification, sharpened frequency selectivity, and compressive nonlinearity (Choe et al., 1998; Camalet et al., 2000; Eguíluz et al., 2000). Even quantitative aspects of hearing accord with the hypothesis that a Hopf bifurcation governs transduction: like the ear's responsiveness, the scaling of responses near this bifurcation follows a power law with an exponent of one-third. The fourth feature of the active process, spontaneous otoacoustic emission, emerges on the unstable side of the Hopf bifurcation, where limit-cycle oscillation occurs.

Electrical engineers discovered in 1914 that operating a regenerative radio receiver near the Hopf bifurcation greatly improves its performance. The ear similarly benefits from this strategy. The utility of amplification is readily evident: there is a selective advantage in responding to the faintest of sounds, in detecting a prey or a predator as early as possible. The importance of the cochlear amplifier becomes painfully apparent when a person becomes "hard of hearing" through failure of the active process. Sharp frequency selectivity is advantageous in that it maximizes the ability to discriminate among similar sounds, be they the rustling of leaves by a moving animal or the subtle inflections of different human dialects. Our ability in this regard is astonishing: an untrained individual can readily distinguish two tones differing in frequency by less than 0.5%, and a trained musician can perform ten times as well. Compressive nonlinearity permits the encoding of a broad range of input intensities by a far narrower gamut of neural firing rates. As a result of the exponent of one-third associated with the power-law responsiveness of the Hopf bifurcation, six orders of magnitude in sound amplitude—and a trillion-fold range of acoustic power—are represented by a variation in neural firing rate of only two orders of magnitude, from a few spikes per second to a few hundred spikes per second. Spontaneous otoacoustic emission presumably occurs, not because it is advantageous per se, but rather as an epiphenomenon associated with the Hopf bifurcation. For optimal performance, the ear must poise itself close to the critical point; negative feedback of a signal representing excitability can be used to achieve this condition (Camalet et al., 2000). Adjusting the control parameter ever so slightly too far, though, yields an oscillation that emerges from the ear as spontaneous otoacoustic emission.

An additional virtue of an active process operating at a Hopf bifurcation is easy phase-resetting behavior. Some oscillators, such as a metronome, are stubborn: they resist efforts to accelerate or retard their movements. Such behavior would be deleterious in an auditory receptor, which must latch onto an unanticipated acoustic stimulus, regardless of its phase, as swiftly as possible. A Hopf oscillator does just that, rapidly resetting its phase to match with that of an external stimulus. All told, operation of the ear's active process at a Hopf bifurcation offers such advantages that it would be surprising if evolution had not adopted that solution.

Active Hair-Bundle Motility

The recognition that a Hopf bifurcation can explain the principal phenomena of auditory transduction has been paralleled by the identification in hair cells of an active process that undergoes just

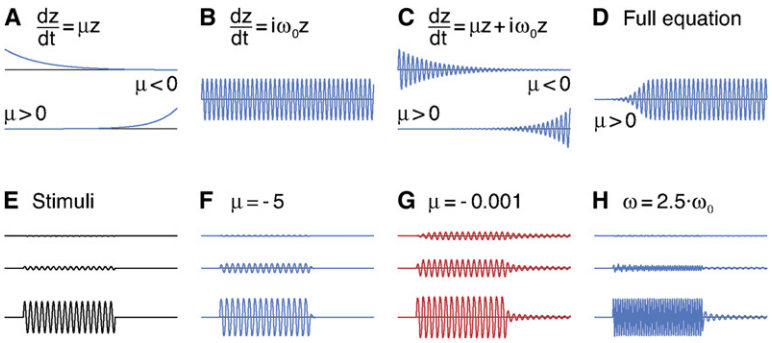

Figure 3. Properties and Consequences of the Hopf Bifurcation

A dynamical system that undergoes a Hopf bifurcation can be described by the relation

$$\frac{dz}{dt}=\mu z+i\omega_0 z-|z|^2 z,$$

in which z is a complex variable that represents hair-bundle or basilar-membrane motion. The nature of the system's responses can be appreciated by evaluating successively the contributions of the three terms on its right side.

(A) The real part of the solution for the simplified equation with only the initial term on the right displays exponential decay for negative values of the control parameter μ or exponential growth for positive values.

(B) Including only the second term on the right leads to solutions that are sine and cosine waves at the natural frequency ω_0.

(C) Combination of the initial two terms produces oscillatory solutions that decline or grow exponentially.

(D) For positive values of the control parameter, the complete equation yields spontaneous limit-cycle oscillation at the natural frequency ω_0. This unforced activity may underlie spontaneous otoacoustic emission. The final term on the right has the effect of arresting the exponential growth of the response, thereby limiting the oscillation to a fixed amplitude.

(E) The characteristics of the active process, as shown in the three subsequent panels, emerge from driving a system that undergoes a Hopf bifurcation with stimuli of relative amplitudes 1, 10, and 100 units (top to bottom).

(F) When the dynamical system operates far from the bifurcation, its passive responses at the natural frequency are nearly linear reflections of the three stimuli.

(G) When functioning near the Hopf bifurcation and stimulated at the natural frequency, the same system displays profound amplification of the smallest input and moderate amplification of the middle one. The lesser degree of amplification of successively greater stimuli represents a compressive nonlinearity: successive 10-fold increments in input evoke only 2.3-fold increases in output.

(H) Even at the bifurcation, the system's tuning is evident from the weak amplification of stimuli whose frequency differs from the natural frequency ω_0. As for the previous panel, $\mu = -0.001$.

such a bifurcation. Active motility by hair bundles in the amphibian ear displays all four hallmarks of the active process. Moreover, there are indications that the same mechanism also operates in the ears of reptiles, birds, and even mammals.

Amplification of mechanical inputs has been demonstrated most directly in the frog's sacculus, whose low-frequency responsiveness permits the direct measurement of the work done on a hair bundle by a flexible stimulus fiber. Stimulation of a hair bundle by nanometer-scale sinusoidal movements of the fiber's base often causes the bundle to move a still greater distance, a phenomenon providing strong evidence of amplification (Figure 4A). Moreover, for some frequencies of stimulation, the phase of hair-bundle oscillation leads that of the stimulus, a phenomenon that cannot occur in a passive system (Martin and Hudspeth, 1999). When the dissipation of energy by hydrodynamic drag is taken into account, the stimulus fiber applied to an active bundle is found to produce *negative* work. This implies that the fiber need not push the bundle through the fluid: the

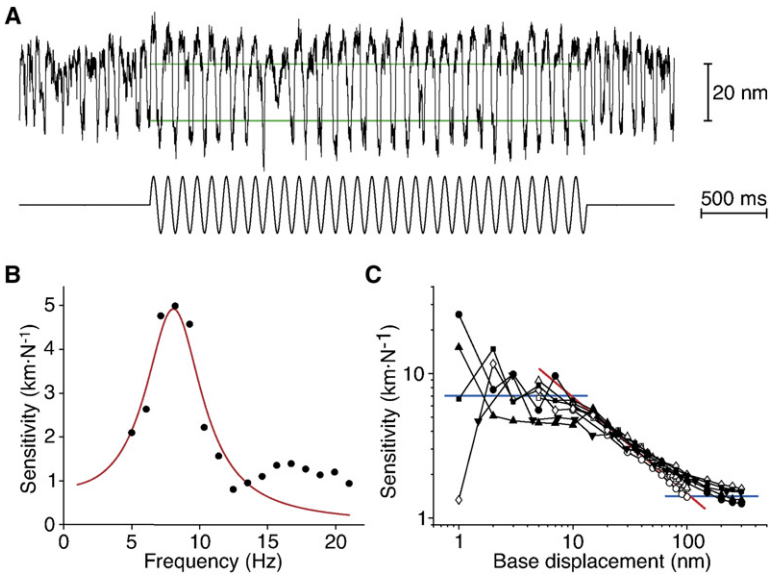

Figure 4. Active Hair-Bundle Motility

(A) The movement of a hair bundle is measured by using a photodiode to detect the displacement of a flexible glass fiber whose tip is attached to the bundle's top. In the absence of stimulation, the hair bundle undergoes irregular oscillations (beginning and end of upper trace) that may underlie the phenomenon of spontaneous otoacoustic emission. When a sinusoidal stimulus of ±10 nm is applied at the fiber's base (lower trace), the hair bundle responds with phase-locked oscillations roughly twice as large; the horizontal green lines demarcate the magnitude of stimulation. This enhanced movement is one indication of a hair bundle's ability to conduct mechanical amplification.

(B) A graph of the sensitivity of a bundle's response as a function of stimulus frequency demonstrates the tuning of the active process. The data points have been fitted by a theoretical relation (red) that characterizes a Hopf oscillator. The hair cells of the frog's sacculus, on which these experiments were conducted, are sensitive to relatively low-frequency seismic and acoustic stimuli.

(C) A doubly logarithmic plot of mechanical sensitivity as a function of stimulus amplitude discloses three regimes of responsiveness at the hair cell's natural frequency. Near threshold, the sensitivity varies almost linearly with the magnitude of stimulation (horizontal blue line at left), but the tiny responses are noisy. For stimuli exceeding 100 nm in amplitude, the responsiveness again approaches linearity as the active process saturates (horizontal blue line at right). Over the intervening range of stimulation, which corresponds to everyday acoustic stimuli, the relation displays power-law behavior with an exponent of −2/3 (oblique red line). This compressive nonlinearity, which is highly consistent for these eight hair bundles, is characteristic of a Hopf bifurcation.

bundle instead pulls the fiber along, unequivocal evidence that the bundle can perform work and amplify its input.

The frequency tuning achieved by a frog's hair cells is modest but clear (Figure 4B). The natural frequencies observed, 5–50 Hz (Martin et al., 2001), lie in the lower range of sensitivity for saccular nerve fibers, 5–130 Hz (Yu et al., 1991). This bias may result from damage during dissection or some deficiency of the in vitro recording environment. In particular, the mechanical load imposed by a stimulus fiber is probably less than that provided in vivo by the otolithic membrane (Benser et al., 1993), and an increased elastic load raises the frequency of oscillation (Martin et al., 2003).

The response of saccular hair bundles to sinusoidal stimulation follows a specific power law indicative of a system operating near a Hopf bifurcation (Martin and Hudspeth, 2001). This is most apparent when the sensitivity of transduction is plotted in doubly logarithmic form against the stimulus strength (Figure 4C). For stimulus amplitudes of no more than a few nanometers, the relation is essentially flat, the signature of linear responsiveness. For stimuli exceeding ±5 nm—corresponding in the mammalian ear to sound-pressure levels common in daily listening—the relation displays a characteristic slope of minus two-thirds. For stimuli above ±100 nm, which represent damaging sound levels, the relation again approaches linearity.

Finally, saccular hair bundles readily produce spontaneous oscillations of the sort expected to underlie spontaneous otoacoustic emissions (Figure 4A; Howard and Hudspeth, 1987; Martin et al., 2003). Because these movements cannot be attributed to Brownian motion (Martin et al., 2001), they signal the performance of work by the bundles against the damping effect of hydrodynamic drag.

Although these experimental results do not prove that saccular hair bundles experience a Hopf bifurcation, the circumstantial evidence is very strong. The data fit every testable prediction generated by the hypothesis, and no contradictory result has emerged. Moreover, modeling suggests that a Hopf bifurcation operates not only in the ears of nonmammalian tetrapods, but in the mammalian cochlea as well. Mating the properties of the bifurcation with those of the cochlear traveling wave produces results that agree well with a variety of experimental observations (Jülicher et al., 2001; Duke and Jülicher, 2003; Kern and Stoop, 2003; Magnasco, 2003).

Mechanical Properties of the Hair Bundle

In sensory receptors that employ a second-messenger cascade for signal amplification, such as photoreceptors and olfactory neurons, an understanding of the transduction process requires a grasp of the intermediate biochemical reactions. To appreciate mechanical amplification by hair cells, one must instead comprehend the behavior of hair bundles by considering successively the contributions of three mechanical elements: stereociliary pivots, gating springs, and transduction channels.

Each stereocilium consists of a rigid rod of actin filaments crosslinked by fimbrin, espin, and perhaps other proteins (Shin et al., 2007). Where the stereocilium tapers at its base, the number of microfilaments diminishes from several hundreds to a few tens that extend as a rootlet into the cellular apex. When a stereocilium is bent at this basal insertion, flexion of this elastic rootlet offers mechanical resistance; taken together, a bundle's ensemble of stereociliary pivots displays a stiffness that is constant for deflection in any direction (Crawford and Fettiplace, 1985; Howard and Ashmore, 1986).

The second mechanical component of a hair bundle is the array of gating springs that sense deflection of the hair bundle. Movement of the bundle in the excitatory direction increases the tension in each gating spring, thus raising the open probability of the associated transduction channel (Corey and Hudspeth, 1983). The tip link, a fine braid of cadherin-23 and protocadherin-15 strands connecting successive stereocilia along the bundle's axis of mechanosensitivity (Kazmierczak et al., 2007), probably forms a portion of each gating spring (Figure 1D; Pickles et al., 1984; Assad et al., 1991). It is likely, though, that other, more compliant elements in series with the tip link also contribute (Kachar et al., 2000). If the transduction channel itself has elastic components, such as the ankyrin repeats of certain TRP subunits, they might constitute the gating spring's principal compliance (Corey et al., 2004; Howard and Bechstedt, 2004). Another possible source of elasticity is the ensemble of myosin molecules to which each transduction channel is thought to be anchored (Howard and Spudich, 1996). Adding gating springs to the hair bundle has the effect of pulling the adjacent stereociliary tips together, and thus of moving the hair bundle in the negative direction. It is for this reason that in the resting bundle the stereociliary pivots are flexed. Just as the tension in a bowstring counters the force produced by a flexed bow, the tension in the gating springs balances the force produced by the bent stereociliary pivots. As would be expected from the orientation of the tip links along the hair bundle's axis of mirror symmetry and of mechanosensitivity, the associated component of stiffness is greatest along that axis (Howard and Hudspeth, 1987).

The gating of transduction channels provides the final component of a hair bundle's mechanical behavior. When a channel opens, the associated gating spring shortens by a few nanometers, reducing the force borne by that spring. This movement introduces into a hair bundle's behavior a nonlinearity that is key to its capacity for performing work.

When mechanical force is applied, for example by a fine, flexible glass fiber (Figure 5A), an ordinary elastic object elongates or shrinks by a distance proportional to the force. This behavior, which is embodied by Hooke's law, is captured in a linear displacement-force relation; the slope of the relation at any point is the stiffness of the elastic object. When a healthy hair bundle is tested in conventional saline solution, though, its displacement-force relation departs from linearity (Figure 5B). For large movements in either the positive or the negative direction, the stiffness is essentially constant. Over a range of about ±20 nm centered at the bundle's resting position, however, the stiffness decreases (Howard and Hudspeth, 1988; Géléoc et al., 1997; Ricci et al., 2000). This decline in stiffness—or increase in compliance—is termed gating compliance because several results associate it with the gating of transduction channels. First, the range of diminished stiffness corresponds to that of mechanoelectrical transduction and shifts with mechanosensitivity during the course of adaptation (Howard and Hudspeth, 1988). Next, the decline in stiffness is abolished by breaking the tip links, leaving an inert hair bundle. Finally, exposure of a bundle to

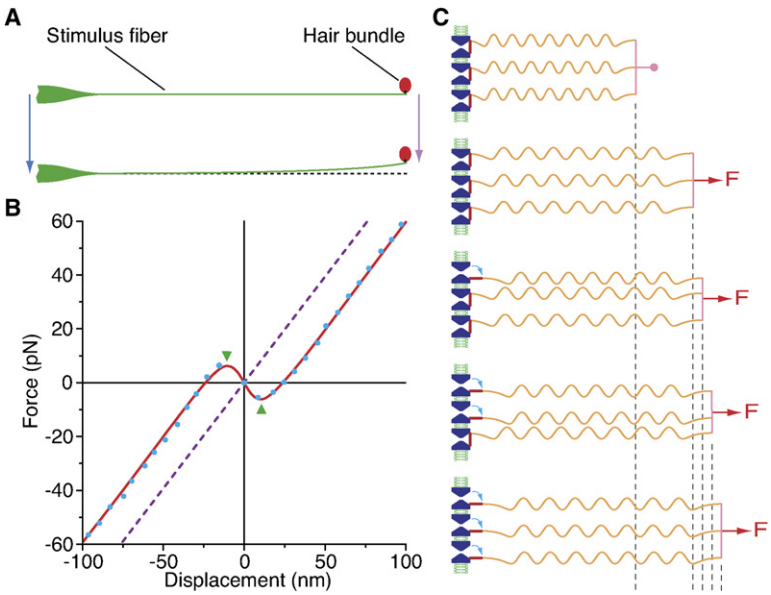

Figure 5. Negative Hair-Bundle Stiffness

(A) The mechanical properties of a hair bundle are assessed by connecting its top to the tip of a glass stimulus fiber 100 μm in length. When a piezoelectrical stimulator displaces the base of the fiber (blue arrow at left), the bundle moves through a lesser distance owing to its stiffness (pink arrow at right). Here, the movements have been exaggerated about 100-fold. By measuring the fiber's flexion and knowing its elasticity, an experimenter can deduce the force exerted by the hair bundle. In a displacement-clamp experiment, negative feedback holds the bundle in a commanded position while the corresponding force is recorded.

(B) The displacement-force relation obtained from a spontaneously oscillating hair bundle (blue points and fitted red curve) differs strikingly from that of a linearly elastic object that obeys Hooke's Law (dashed purple line). Over the range of positions between the green arrowheads, the hair bundle displays negative stiffness; the unrestrained bundle cannot remain in this region, but must leap spontaneously in the positive or negative direction.

(C) The coordinated gating of mechanoelectrical-transduction channels explains the hair bundle's negative stiffness. In this representation, three channels from distinct stereocilia are depicted in a common membrane in the interest of compactness (first diagram). When a constant force *F* is applied to the parallel array of channels, the three gating springs are stretched to an identical extent (second diagram). As one channel opens, the movement of its gate partially relaxes the associated gating spring (third diagram). Because the gating springs for the two remaining channels consequently bear additional tension, either of those channels is more likely to open as well (fourth diagram). This phenomenon continues until all three channels have opened (fifth diagram). The system is bistable: it can dwell in a configuration in which no channels are open or one in which all are ajar, but is unstable at the intermediate positions.

aminoglycoside antibiotics, which reversibly block the transduction channels (Kroese et al., 1989; Marcotti et al., 2005), also eliminates the stiffness change (Howard and Hudspeth, 1988; Martin et al., 2003).

Gating compliance is an indication of the direct nature of mechanoelectrical transduction by hair cells, whose hallmark is reciprocity (cf. Markin and Hudspeth, 1995; Hudspeth et al., 2000). The application of force to a gating spring changes the probability of channel gating; an increased force, for example, promotes opening. At the same time, channel gating affects the tension in a gating spring; thus, channel opening relaxes the spring. The mechanical effects of channel gating are therefore expected only when channels are able to make a transition between the closed and open states.

The linchpin of amplification by active hair-bundle motility is an extraordinary mechanical feature of the hair bundle: negative stiffness (Martin et al., 2000). When a hair cell is placed in the unique ionic environment of the inner ear, with its hair bundle bathed in low-Ca^{2+} endolymph, the magnitude of the gating compliance can equal or exceed the combined stiffnesses of the gating springs and stereociliary pivots (Denk et al., 1992; cf. Markin and Hudspeth, 1995; Hudspeth et al., 2000). Under these conditions, and over a specific range of displacements, the slope of the displacement-force relation—the hair bundle's stiffness—becomes zero or even negative (Figure 5B).

What is the meaning of negative stiffness? Positive stiffness is characterized by the observation that an object opposes externally applied force: a pushed object pushes back. By contrast, an object displaying zero stiffness offers no resistance to an imposed force, in principle moving an indefinite distance in response. Still more strangely, an object with negative stiffness produces additional force *in the same direction* that it is displaced by an external stimulus. In the instance of a hair bundle, negative stiffness is manifested when the bundle's top moves farther than the base of the flexible stimulus fiber used to apply force.

Negative stiffness emerges from interactions among the transduction channels (Figure 5C). Because these channels lie essentially in parallel (Jacobs and Hudspeth, 1990; Iwasa and Ehrenstein, 2002; Kozlov et al., 2007), an external force applied to a hair bundle is distributed among them. If all of the channels are in the same state, for example closed, the force is divided equally among the associated gating springs. Suppose a single channel now opens, reducing in part the tension in its gating spring. The remaining springs, which must accommodate an increased load, bear more tension than originally, and the associated channels are more likely to open. The more channels that have opened, in other words, the greater is the load on the balance. For a particular range of positions, corresponding to a specific range of open probabilities, opening an additional channel triggers an avalanche in which the remaining channels open in concert. A similar argument holds for channel closure: once a significant fraction of the channels has shut, the remainder tend to close in unison. As a surge of channel opening or closing drives the hair bundle in respectively the positive or the negative direction, the bundle can push or pull against an external load—the basis of negative stiffness, and the substrate for active hair-bundle motility.

Myosin Motors and Active Hair-Bundle Motility

Although the instability fostered by concerted channel gating is a necessary ingredient for active hair-bundle motility, it must be coupled to an energy source in order to do work. The molecular motors responsible for adaptation of mechanoelectrical

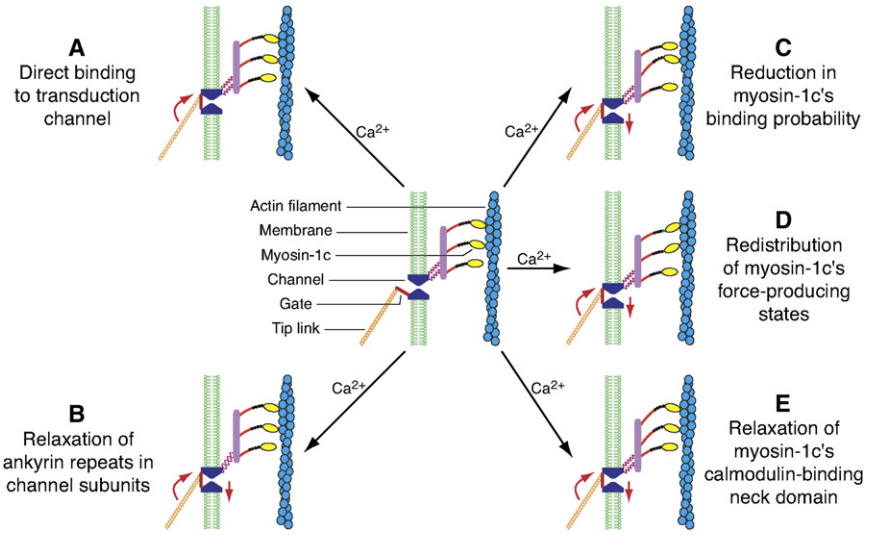

Figure 6. Possible Mechanisms of Ca^{2+}-Induced Reclosure of Transduction Channels

When mechanical stimulation tenses the tip link, a transduction channel opens as shown in the central diagram.

(A) Ca^{2+} entering through an open transduction channel might bind to some component of the channel itself; the binding energy would then close the channel's gate. This arrangement would have the effect of increasing the tension in the associated tip link.

(B) If the transduction channel is a member of the TRP family, it might be anchored by ankyrin repeats whose relaxation in the presence of Ca^{2+} would allow the channel to slip downward, reducing the tension in the tip link and thus closing the channel.

(C) Ca^{2+} might diminish the probability that myosin-1c molecules are bound to cytoskeletal actin filaments, thus allowing the transduction element to move downward and the channel to reclose.

(D) The accumulation of Ca^{2+} might reduce tip-link tension by favoring a backward step by myosin-1c molecules.

(E) The binding of Ca^{2+} to calmodulin molecules adorning the IQ domains might relax the neck region of myosin-1c molecules and thereby allow the channel to move downward. Because the myosin heads would not detach from actin filaments in the last two instances, those mechanisms could potentially underlie the active process even at high stimulus frequencies.

transduction evidently power the active process as well. The sensitivity of hair cells is so great that the transduction apparatus can be saturated by stimuli only a few tens of nanometers in amplitude. To prevent saturation as a result of steady hair-bundle offsets, transduction displays a unique form of adaptation that continually resets a bundle's range of sensitivity to accord with the position at which the bundle is held (Eatock et al., 1987; cf. Hudspeth and Gillespie, 1994; Gillespie and Corey, 1997; Eatock, 2000; LeMasurier and Gillespie, 2005). This adaptation is mechanical in nature, for the force produced by a hair bundle changes as adaptation proceeds (Howard and Hudspeth, 1987; Jaramillo and Hudspeth, 1993; Ricci et al., 2000; Kennedy et al., 2005), the bundle moves when the degree of adaptation is altered (Assad et al., 1989; Assad and Corey, 1992), and the region of negative stiffness migrates during adaptation (Martin et al., 2000; LeGoff et al., 2005).

A wealth of evidence suggests that adaptation is effected by a mechanoenzyme, and more specifically by one or more isoforms of myosin (Howard and Hudspeth, 1987; Gillespie et al., 1993). Every stereocilium is packed with actin filaments, for which the only known motors are of the myosin family. Adaptation is arrested by nucleoside diphosphates and inorganic phosphate analogs that interfere with myosin's ATPase cycle (Gillespie and Hudspeth, 1993; Yamoah and Gillespie, 1996). Of at least five types of myosin known to occur in the hair bundle, only myosin-1c is concentrated where adaptation is thought to occur, near the insertional plaque at the upper end of each tip link (Gillespie et al., 1993; García et al., 1998; Steyger et al., 1998). The most compelling evidence for a role of myosin-1c stems from the transgenic expression of protein altered by site-directed mutagenesis. Replacement of the bulky tyrosine residue covering the nucleotide-binding cleft with a smaller glycine residue permits N^6(2-methyl butyl)-ADP and -ATP, molecules too large for wild-type myosin-1c to bind, to enter the cleft. When a hair cell from a transgenic mouse bearing this alteration is exposed to the bulky ADP analog, adaptation is arrested (Holt et al., 2002). Still more importantly, the hair cells of knockin animals homozygous for the mutation can perform adaptation using the ATP derivative (Stauffer et al., 2005). It follows that myosin-1c is almost certainly a component of the adaptation motor. Because mutants lacking myosin VIIa display abnormalities of adaptation (Kros et al., 2002), however, it remains unclear whether myosin-1c is the only isoform involved.

A second mechanism of active force production in the hair bundle involves Ca^{2+}-dependent reclosure of transduction channels. Pushing a hair bundle in the excitatory direction elicits a transduction current that rapidly peaks but then declines within milliseconds toward a plateau. Concomitantly with this fast adaptation, the hair bundle exerts force in the direction opposite that of the stimulus (Howard and Hudspeth, 1987; Benser et al., 1996). Ion-substitution experiments have attributed this response to Ca^{2+}-dependent reclosure of the transduction channels, a phenomenon now known for hair cells from amphibians, reptiles, and mammals. The molecular basis of Ca^{2+}-dependent channel reclosure remains uncertain. It was originally proposed that the energy associated with the binding of Ca^{2+} to the channel or an associated protein directly reduces the channel's open probability (Figure 6A; Corey and Hudspeth, 1983; Crawford et al., 1991; Cheung and Corey, 2006). Mechanical measurements suggest, however, that Ca^{2+} binding instead relaxes some elastic element that then permits channel reclosure (Bozovic and Hudspeth, 2003; Martin et al., 2003). If the transduction channel proves to be a member of the TRP family, many of which contain extensive chains of ankyrin repeats, extension of those anchoring segments in response to Ca^{2+} could implement the relaxation (Figure 6B; Corey et al., 2004; Howard and Bechstedt, 2004).

More recent evidence suggests that the site of relaxation lies in the adaptation motors (Bozovic and Hudspeth, 2003; Martin et al., 2003; Stauffer et al., 2005). The binding of Ca^{2+} might

simply reduce the probability that myosin-1c molecules are bound to actin, thus allowing the transduction element to sag downward along the stereocilium (Figure 6C). Another attractive hypothesis is that Ca^{2+} alters the equilibrium between two bound states of myosin-1c molecules (Figure 6D). As in the instance of insect flight muscle, this could occur without the necessity of myosin's detachment from its actin substrate, permitting oscillation well into the kilohertz range (Hudspeth and Gillespie, 1994). Although myosin-1c exhibits only torpid movements in conventional assays of in vitro motility (Gillespie et al., 1999), it remains unclear how quickly the molecule can rock between its actin-bound states (Batters et al., 2004a, 2004b). Finally, the calmodulin-binding neck domain of myosin-1c might relax upon Ca^{2+} binding (Figure 6E; Howard and Spudich, 1996). The Ca^{2+} occupancy of calmodulin molecules somehow transmits along the neck of a myosin molecule a signal that regulates motor activity; perhaps that signal involves a change in the shape or stiffness of the neck that also mediates fast adaptation.

A key point about four of the proposed mechanisms for Ca^{2+}-dependent channel reclosure (Figures 6A, 6B, 6D, and 6E) is that the gradient of Ca^{2+} concentration across the hair cell's membrane, rather than the hydrolysis of ATP, actually powers the process on a cycle-by-cycle basis (Choe et al., 1998). In each instance, the binding energy of Ca^{2+} to the channel, to its cytoskeletal anchorage, or to associated myosin-1c molecules is used to perform external work on a stimulus fiber or against hydrodynamic drag. By this model, myosin-1c might display two types of motility with distinct mechanisms, energy sources, and timescales. Slow adaptation would represent conventional myosin-based motility, powered by ATP and proceeding at a rate limited by two slow steps, the stereospecific binding of nucleotide and the docking of myosin heads to actin filaments. Fast adaptation would instead occur through a structural rearrangement, driven by Ca^{2+} entry into the cytoplasm and able to operate much more swiftly because Ca^{2+} binding is diffusion-limited and the relevant myosin heads remain engaged with actin throughout the process.

The strongest link between hair-bundle mechanics, myosin activity, and the active process is provided by modeling studies. The extensive description of the hair bundle's properties provided by numerous experiments permits a detailed quantitative mathematical representation of the bundle (cf. Howard et al., 1988; Markin and Hudspeth, 1995). Adaptation can be modeled on the assumption that the upper attachment of each tip link is subject to two forces: the downward tension in the tip link and the upward force exerted by the myosin-based motor (Assad and Corey, 1992). Combination of these two models reveals how the essential elements interact to yield the characteristics of the active process (Figure 7; Bozovic and Hudspeth, 2003; Martin et al., 2003; Nadrowski et al., 2004; cf. Martin, 2007).

Mathematical analysis of Ca^{2+}-dependent channel reclosure not only reproduces many features of the active process but also led to the first realization that a Hopf bifurcation can explain the ear's characteristics. A model that invokes Ca^{2+}-dependent reclosure of transduction channels yield amplification, tuning, compressive nonlinearity, and the capacity for spontaneous oscillation (Choe et al., 1998). The model produces active and passive behaviors over the full frequency range of human audition through variation in the values of only two parameters. One is the number of stereocilia, which is known to vary along the tonotopic gradients of many cochleas in association with systematic variations in stereociliary length (Tilney and Saunders, 1983). The second variable parameter, the activation energy of channel gating, requires that transduction channels along an array of hair cells somehow be tuned to different frequencies. There is precedent for such tuning, though: the properties of the Ca^{2+}-sensitive K^+ channels responsible for electrical resonance in hair cells are adjusted along the tonotopic gradient by alternative splicing of the cognate mRNA (Navaratnam et al., 1997; Rosenblatt et al., 1997; Ramanathan et al., 1999). Other parameters are known to be adjusted as well: for example, the turtle's cochlea exhibits tonotopic gradients in the conductance of transduction channels and in the concentration of proteinaceous Ca^{2+} buffers (Ricci et al., 2003; Hackney et al., 2005).

The simulated responses of the model to several pharmacological manipulations also accord with experimental observations. Drugs that increase cAMP-dependent phosphorylation of proteins, perhaps including myosin-1c, lead both in models and in single-cell experiments to larger, slower bundle oscillations (Martin et al., 2003). Pharmacological manipulations that reduce phosphorylation have the opposite effect. Finally, butanedione monoxime, which interferes with myosin activity by an uncertain means that may involve protein dephosphorylation, arrests spontaneous hair-bundle movements altogether.

The Origin of the Active Process

The presence of active hair-bundle motility in frogs, turtles, birds, and mammals implies that this component of the active process evolved no later than the origin of tetrapods some 360 million years ago. The mechanism is perhaps still more ancient, however, and may have antedated the origin of the craniate hair bundle. Although the auditory receptor organs of dipteran insects are structurally quite distinct from those of vertebrates, their mechanosensitive chordotonal receptors employ an active process with remarkably similar properties. In fact, the model developed to describe active hair-bundle motility (Nadrowski et al., 2004) fits the dipteran system with minimal alterations (Albert et al., 2007).

It is possible to describe a theoretical schema whereby active hair-bundle motility might have evolved in three stages. First, the coupling of some type of ion channel to the cytoskeleton and to external stimuli produced a mechanoreceptive cell. This event conferred the selective advantage of rapid transduction (Corey and Hudspeth, 1979) but at the same time imposed the nonlinearity associated with the gating compliance of transduction channels (cf. Hudspeth et al., 2000). As more channels participated in the response, and as their mechanical arrangement became more nearly parallel, the sensitivity of the mechanoreceptor rose, narrowing its range of responsiveness, and the gating compliance increased toward the point of negative stiffness. In a second evolutionary step, the augmented sensitivity of the transduction apparatus necessitated the development of an adaptation mechanism—based on myosin in the instance of vertebrates—to maintain the transduction apparatus near its position of greatest sensitivity. With the substrates of negative stiffness and powered adaptation in place, the final step involved the interaction of the two to achieve active hair-bundle motility and

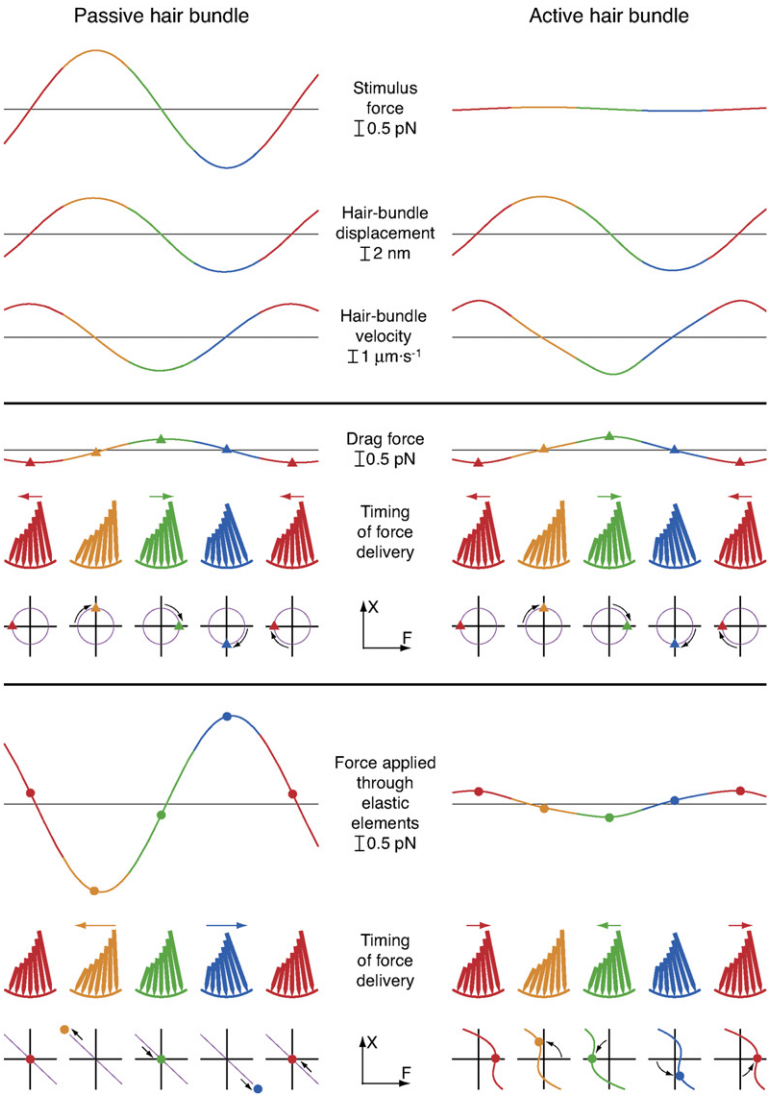

Figure 7. The Mechanism of Amplification by Active Hair-Bundle Motility

Two simulations depict the responses of hair bundles to 80 Hz sinusoidal stimulus forces that produce hair-bundle displacements of ±5.7 nm. The color coding in each panel distinguishes successive phases of the simulation. To obtain the response from a passive hair bundle (left) requires a stimulus force of ±3 pN, whereas the active hair bundle (right) needs only ±0.1 pN. In this example, then, the active process confers an amplification of 30×. The open probability of the transduction channels correspondingly varies between 0.3 to 0.7 in the presence of the active process, but changes by only 0.1% in the passive circumstance. Because the hair bundles follow similar trajectories in both the passive and the active instances, the temporal traces of the hair-bundle velocity are similar. Plots of the drag force, which is proportional to bundle velocity, also show nearly identical responses for the two conditions. The cartoons indicate that movement of the bundle toward the right, the positive direction, is associated with a drag force in the opposite direction (red arrows). Leftward bundle motion conversely elicits a drag force toward the right (green arrow). The five small graphs, which display the successive relations between drag force (*F*) and hair-bundle position (*X*), reveal that the passive and active responses both follow clockwise trajectories indicative of energy dissipation. For the passive hair bundle, the force applied through two types of elastic elements, the gating springs and the stereociliary pivots, is quite large. The cartoons show that extreme deflection to the right evokes a maximal elastic restoring force to the left (orange arrow), whereas peak displacement to the left elicits the greatest rightward force (blue arrow). The force-displacement graphs below the cartoons indicate that the passive hair bundle displays linear elasticity: the restoring force follows Hooke's law. Despite its magnitude, the force applied through elastic elements is out of phase with the drag force and cannot cancel it. The stimulus must therefore supply at least 7 zJ of energy during each cycle of oscillation to overcome viscous dissipation. Although the displacement of the active hair bundle closely resembles that for the passive bundle, the active process greatly alters the magnitude, and especially the timing, of the force applied through elastic elements. As the cartoon demonstrates, the greatest force to the right occurs as the hair bundle moves most quickly in that direction (red arrows), and the strongest force to the left arises during the fastest leftward motion (green arrow). The graphs beneath the cartoons reveal how this result emerges from the two components of the active process. First, adaptation continuously shifts the force-displacement relation back and forth (colored curves). And second, each excursion across the unstable region of negative stiffness speeds the hair bundle's motion. As a consequence, the operating point describes a counterclockwise trajectory nearly equal and opposite that of the drag force. In other words, whereas for the passive hair bundle the force delivered through elastic elements is in phase with bundle *displacement*, for the active bundle the corresponding force is in phase with bundle *velocity*. The stimulus does almost no work because the active process delivers an amount of energy equivalent to that lost to drag. The active process thus acts as "negative viscosity," countering the inevitably dissipative effect of the viscous medium through which the hair bundle moves. The equations used in the simulations are those of Martin et al. (2003).

confer the selective advantages detailed earlier. This progression seems straightforward enough that it might have taken place early in the history of mechanotransduction or could have occurred independently in the protosotome and deuterostome lineages. Over the next few years, completion of the molecular description of the transduction apparatus in flies and vertebrates may reveal which evolutionary trajectory actually transpired.

The Control Parameter for Active Hair-Bundle Motility

It is characteristic of a system displaying a Hopf bifurcation that one or more control parameters determine whether the system is quiescent or oscillates spontaneously (cf. Strogatz, 1994). Although no control parameter for active hair-bundle motility has yet been identified, several lines of evidence suggest that activity is regulated by Ca^{2+} within stereocilia. Raising the extracellular Ca^{2+} concentration, and thus allowing more Ca^{2+} to enter the cell, slows and eventually suppresses spontaneous bundle oscillation (Martin et al., 2003; Tinevez et al., 2007). Reducing the extracellular Ca^{2+} concentration with chelators instead accelerates oscillation. And passing electrical current across the sensory epithelium, which alters the flow of Ca^{2+} into hair cells, has effects consistent with the foregoing (Bozovic and Hudspeth, 2003).

The key role of Ca^{2+} in regulating oscillation implies that other sources of the ion may supplement transduction channels to determine the state of the active process. Ca^{2+} traverses the basolateral plasmalemma through L-type channels activated by depolarizing receptor potentials (Hudspeth and Lewis, 1988; Zidanic and Fuchs, 1995). Although much of this Ca^{2+} is

extruded by pumps or captured by the endoplasmic reticulum (Tucker and Fettiplace, 1995; Issa and Hudspeth, 1996; Yamoah et al., 1998; Hill et al., 2006), strong stimuli may admit sufficient Ca^{2+} to affect the concentration in the hair bundle. Desensitization of the active process owing to Ca^{2+} entry would constitute negative feedback to the transduction apparatus.

The active process might also be downregulated by activation of a hair cell's efferent innervation, which mediates Ca^{2+} influx through acetylcholine receptors comprising α9 and α10 subunits (Katz et al., 2000; Weisstaub et al., 2002). Because Ca^{2+} would have to diffuse from the base of the hair cell to the top of the hair bundle, this process could also provide feedback on a slow timescale of hundreds of milliseconds. Activation of the efferent innervation additionally evokes a hyperpolarization (Art et al., 1982) that immediately increases the driving force for Ca^{2+} entry through transduction channels and thereby more rapidly affects the bundle's ability to oscillate. The Ca^{2+} permeability of the P2X purinergic receptors in the stereociliary membrane implies that their activation by extracellular ATP would afford another means of introducing Ca^{2+} (Ashmore and Ohmori, 1990; Raybould and Housley, 1997). Finally, the signals measured in kinocilia with Ca^{2+}-sensitive fluorophores and the binding of dihydropyridines to the kinociliary plasmalemma suggest that kinocilia provide an additional avenue for Ca^{2+} entry that could modulate the active process (Denk et al., 1995; Boyer et al., 2001).

The Influence of Hair Bundles on Basilar-Membrane Motion

Are the exertions of hair bundles sufficient to affect basilar-membrane motion, especially in the mammalian cochlea? This might seem implausible, for the basilar membrane is a macroscopic strip of connective tissue surmounted by numerous cells, of which the hair cells constitute only a minority. At first glance, then, hair bundles seem too flimsy to influence the movement of the basilar membrane. When assessed with an in vitro preparation of the mammalian cochlea that retains its active process, however, hair bundles make an unexpectedly large contribution to the stiffness of the cochlear partition (Chan and Hudspeth, 2005b). Moreover, even when the active process has been abolished, the nonlinearity associated with gating compliance is measurable in distortion-product otoacoustic emissions (Liberman et al., 2004).

It must also be borne in mind that the cochlea's active process is most important at the natural frequency of each segment of the basilar membrane, the frequency at which that segment resonates. Although the basilar membrane is more elaborate than a simple mechanical resonator, its fundamental components are similar: elasticity, characterized by the stiffness κ; inertia, denoted by the mass m; and viscous damping, signified by the drag coefficient ξ. The mechanical impedance, or resistance to motion, afforded by such a structure during stimulation at an angular frequency ω has a magnitude

$$|Z| = \left|\frac{F}{v}\right| = \sqrt{\xi^2 + \left(\omega m - \frac{\kappa}{\omega}\right)^2} = \sqrt{\xi^2 + \left(\omega^2 - \omega_0^2\right)^2 \left(\frac{m}{\omega}\right)^2},$$

in which F is the amplitude of the sinusoidal force stimulus and v the ensuing velocity of the basilar membrane. Resonance occurs at ω_0, the system's natural frequency, which is specified by $\omega_0 = \sqrt{\kappa/m}$. At that frequency, the influence of the mass cancels that of the stiffness. Because it essentially vanishes at resonance, the basilar membrane's stiffness does not preclude the possibility that hair bundles amplify basilar-membrane movement. $Z = \xi$ at resonance, so the critical issue is instead whether the bundles can produce forces sufficient to counter the effect of viscosity. This is quite plausibly so. A 10 μm long segment of the basilar membrane, which contains three outer hair cells and a single inner hair cell, has a drag coefficient of ~120 $nN \cdot s \cdot m^{-1}$ (Ospeck et al., 2003). When stimulated at 10 kHz through a distance of ±3 nm, corresponding to a sound-pressure level near 60 dB, each outer hair cell would have to counter a peak drag force of 8 pN. Individual anuran hair bundles—which are considerably smaller than those of a mammal—can produce active forces more than twice that great (Le Goff et al., 2005), so the hair bundles in a cochlear segment are potentially able to overcome the viscous drag.

Membrane-Based Electromotility in the Mammalian Cochlea

Although the active process in the ears of nonmammalian tetrapods is almost certainly based on active hair-bundle motility, the situation is far less clear for mammalian hearing. The problem is not a lack of candidates for the active process, bur rather uncertainty about the relative contributions to the ear's performance of two mechanisms: active hair-bundle motility and membrane-based electromotility (cf. Hudspeth, 1997; Fettiplace and Hackney, 2006).

When an outer hair cell isolated from the mammalian cochlea is electrically stimulated, the cell body undergoes a striking change in shape (Brownell et al., 1985). Depolarization shortens the cell; because the cytoplasmic volume is conserved, this movement is accompanied by an increase in diameter. Hyperpolarization conversely evokes lengthening and narrowing. Electromotility can cause length changes as great as 4% and can occur at frequencies as great as 25–80 kHz (Gale and Ashmore, 1997; Frank et al., 1999), an attractive feature for a candidate active process required to operate at high frequencies. Because the characteristics of electromotility have recently been reviewed in extenso (cf. Ashmore, 2008), further details will be omitted here.

Electromotility is effected by the protein prestin, a member of the sulfate-transporter family, of which some ten million copies exist in paracrystalline rafts in the lateral plasmalemma of an outer hair cell. Although these arrays are associated with cytoplasmic pillars and often with cisternae of smooth endoplasmic reticulum, those features are not required for motility (Holley and Ashmore, 1988). Nor does electromotility require ATP or another source of chemical energy. Changes in cellular length are instead controlled by the transmembrane electrical field: depolarization evidently decreases the surface area of the membrane occupied by each prestin molecule, allowing the cell to shorten; hyperpolarization has the opposite effect. The phenomenon is marked by nonlinear membrane capacitance that reflects the prestin molecule's structural rearrangement over a particular range of membrane potentials, a phenomenon analogous to

the movement of gating charge in voltage-sensitive ion channels (Santos-Sacchi, 1991; Iwasa, 1993; Gale and Ashmore, 1994).

An impressive body of evidence, most of it obtained from transgenic mice, implicates membrane-based electromotility in the active process of the mammalian cochlea. Knocking out the prestin gene produces a severe decrease in the sensitivity of hearing (Liberman et al., 2002; Cheatham et al., 2004), but the interpretation of this result is complicated. Basilar-membrane movement in knockout animals displays normal sensitivity but lacks nonlinearity (Mellado Lagarde et al., 2008a). It appears that the absence of prestin, which produces smaller and softer outer hair cells (Jensen-Smith and Hallworth, 2007), frees the basilar membrane from the encumbrance of the organ of Corti but at the same time disrupts the transmission of mechanical stimuli from the basilar membrane to the mechanosensitive hair bundles. Moreover, the transgenic animals soon lose the outer hair cells—and even the inner hair cells, which are not thought to express prestin—at the cochlear base (Liberman et al., 2002; Cheatham et al., 2004). It is unclear whether this cell death is an artifact of the animals' genetic background or signals the fact that prestin retains an essential function such as ion translocation (Muallem and Ashmore, 2006).

Clearer results emerge from a study in which prestin's voltage sensitivity is shifted so radically that the molecule is locked in one conformational state. Although the voltage sensors for electromotility remain uncertain (Oliver et al., 2001; Rybalchenko and Santos-Sacchi, 2003), mutations of specific amino acids in prestin can alter the molecule's responsiveness to electrical stimuli. It has therefore been possible to produce transgenic knockin mice whose outer hair cells contain normal amounts of prestin of essentially native structure, but of negligible voltage sensitivity (Dallos et al., 2008). The outer hair cells in these mutants display essentially normal passive mechanical properties and presumably retain active hair-bundle motility, but the animals evince severe hearing impairment indicative of abolition of the active process.

Another strong line of support for a role of electromotility comes from investigation of electrically evoked otoacoustic emissions, which are thought to reflect activation of the active process by externally applied electrical current. The deflection of hair bundles by shearing motions of the tectorial membrane normally represents a significant part of the stiffness loading the basilar membrane (Chan and Hudspeth, 2005b). When the hair bundles are decoupled from the tectorial membrane by mutation of α-tectorin, a protein essential to the tectorial membrane's integrity (Legan et al., 2000), the animals become profoundly deaf. Because electrical stimulation still evokes normal acoustic emissions, however, membrane-based electromotility evidently accounts for the effect of electrical stimulation without a contribution from the hair bundles (Mellado Lagarde et al., 2008b).

Despite our detailed knowledge of the biophysical basis of membrane-based electromotility, and notwithstanding the circumstantial evidence for a role of the phenomenon in cochlear amplification, it remains uncertain *how* electromotility contributes to the active process. Electromotility has not been demonstrated at a single-cell level to produce amplification, tuning, or compressive nonlinearity. Moreover, it is unclear what energy source might be coupled to electromotility to evoke basilar-membrane oscillations and thus account for spontaneous otoacoustic emissions. It is conceivable that prestin has a role essential to the active process, but does not itself account for the four cardinal features of the process. One possibility is that electromotility tunes the basilar membrane (Kim, 1986; Kennedy et al., 2005). For active hair-bundle motility to operate effectively, its kinetics must be closely matched to the natural frequency of the associated basilar membrane. If developmental processes cannot produce this matching with sufficient precision, the stiffness change associated with electromotility (He and Dallos, 2000) might adjust the resonance of each increment of the basilar membrane much as twisting a peg in a violin's pegbox tunes a string. This mechanism would be admirably matched to the ear's efferent control system: the firing of efferent fibers could hyperpolarize outer hair cells, shifting their stiffness and thus detuning the local resonance. Although a knockin experiment involving prestin with an altered voltage response excludes certain types of basilar-membrane tuning (Gao et al., 2007), others remain possible.

A persistent difficulty in understanding the role of electromotility in amplification is the membrane time constant. The resistance and capacitance of an outer hair cell imply that a voltage-driven process such as electromotility should diminish in sensitivity above a corner frequency of only a few hundred hertz, rendering electromotility quite inefficient in the upper range of mammalian audition (Santos-Sacchi, 1992; Gale and Ashmore, 1997). Four interesting proposals have been broached as potential solutions to this problem. First, membrane-based electromotility might be driven by an extracellular voltage change, the cochlear microphonic potential, whose effects would not be subject to the membrane time constant (Dallos and Evans, 1995). Next, electromotility might operate in conjunction with exceptionally speedy voltage-gated ion channels to form a system capable of undergoing a Hopf bifurcation and thus of explaining the active process (Ospeck et al., 2003). A third hypothesis is that the piezoelectrical nature of electromotility confers upon outer hair cells properties not expected of circuits containing only resistive and capacitive elements (Weitzel et al., 2003). Finally, prestin's changes in shape might be gated, not directly by membrane potential, but instead by the local concentration of Cl^- immediately inside the plasmalemma (Rybalchenko and Santos-Sacchi, 2003). Unfortunately, none of these ingenious suggestions has yet been validated by experimentation. An even more radical possibility is that electromotility participates in forward mechanoelectrical transduction at high frequencies. Because prestin's piezoelectric effect is reversible (Gale and Ashmore, 1994), each compression of an outer hair cell during upward movement of the vibrating basilar membrane should cause a hyperpolarization whose timecourse is not restricted by the membrane time constant and that could effect amplification by driving hair-bundle motility electrically (Bozovic and Hudspeth, 2003).

Another reason for the uncertainty about the nature of the mammalian active process is the evidence that active hair-bundle motility persists in mammals. The nonlinearity owing to gating compliance is evident in distortion-product otoacoustic emissions, even in prestin-knockout animals (Liberman et al., 2004). Electrically evoked otoacoustic emissions, which in

nonmammalian species likely stem from hair-bundle movements (Bozovic and Hudspeth, 2003), also endure partially in knockout mice (Drexl et al., 2008). In vitro recordings from normal cochleas suggest that both electromotility and hair-bundle motility mediate the response to electrical stimulation (Chan and Hudspeth, 2005b; Kennedy et al., 2006). The strongest evidence emerges from an in vitro preparation of the mammalian cochlea, which displays amplification, tuning, and compressive nonlinearity that vanish reversibly when the endocochlear potential is removed or the transduction channels are blocked (Chan and Hudspeth, 2005a, 2005b). The preparation affords an opportunity to examine the basis of the active process by varying the ionic composition at the hair cells' apex. In particular, active hair-bundle motility depends upon the flow of Ca^{2+} through transduction channels, but is largely independent of the larger transduction current borne by K^+. Membrane-based electromotility, by contrast, depends directly on the transmembrane potential and therefore on the total transduction current. Because the hallmarks of the active process persist in the presence of an artificial endolymph solution in which K^+ has been replaced by an impermeant cation, active hair-bundle motility appears to mediate at least part of the active process in this mammalian preparation.

Perhaps the most likely possibility is that prestin's activity has in mammals supplemented rather than replaced entirely the phylogenetically ancient process of active hair-bundle motility. It is plausible, for example, that the features of negative stiffness and adaptation persist in the hair bundles of outer hair cells, but that the motor function of myosin-1c has been assumed, in part or in full, by prestin. In the archaic parlance of audio technology, active hair-bundle motility may constitute the system's tuner and preamplifier, whereas electromotility provides the power amplifier. Such a situation would explain why the characteristics of the active process in mammals so closely resemble those in other tetrapods: the mammalian active process has inherited the diagnostic features associated with the Hopf bifurcation. It may also be that electromotility and active hair-bundle motility differ in their influence along the cochlear spiral, with the former predominating at the high-frequency basal extreme and the latter increasingly important toward the low-frequency apex. Indeed, the experiments demonstrating a role for electromotility have been conducted predominantly at the cochlear base, whereas those examining hair-bundle motility refer to the apical and middle cochlear turns.

Conclusion

The remarkable properties of vertebrate hearing stem from the ear's active process, which in turn benefits from functioning near a dynamical instability. Operation of the mammalian active process at a Hopf bifurcation may also explain several features of cochlear responsiveness that have proven problematical. Although conventional models of cochlear amplification require that energy be added to a traveling wave over a substantial distance basal to the point at which the wave peaks, some experiments argue against such an arrangement (Allen and Fahey, 1992; de Boer et al., 2005). The Hopf bifurcation is highly frequency selective, however, so amplification according to that principle can be confined to the immediate vicinity of the peak (Duke and Jülicher, 2003; Magnasco, 2003); there need be no conflict between theory and experiment. Although experimental estimation of the energy flow along a traveling wave suggests that there is little or no power gain, an active process based on the Hopf bifurcation does not require a net power gain. The basilar membrane and the hair cells above it work best when they are most resonant, when they can accumulate energy over many cycles of stimulation. Viscous damping, which dissipates stimulus energy, is the enemy of resonance. Just around the bifurcation, the active process can improve hearing by counteracting the energy dissipation associated with viscous drag on the vibrating cochlear partition. In other words, the effect of the active process is not so much the addition of energy to a dissipative basilar membrane as the effective abolition of the dissipation itself.

Future progress in understanding the active process, whether in mammals or in other tetrapods, will focus on the molecular substrates of the observed phenomena. Three approaches seem especially promising. First, the growing use of transgenic mice offers opportunities to determine the roles of the panoply of hair cell-specific proteins. Genetic studies have identified numerous proteins that are expressed dominantly or exclusively in hair cells and whose activity is essential for the ear's normal function (cf. Petit, 1996). Investigation of transgenic models for the hundred or so forms of nonsyndromic deafness, as well as of deaf mice produced in mutagenic screens, should disclose the functions of many of these proteins, some of which are likely to be involved in the active process. Because several techniques exist for the regulated expression of transgenes, it should be possible in these experiments to circumvent the problem of hair-cell death associated in the long term with some mutant phenotypes. A similar approach should further clarify the contributions of known proteins, such as prestin. As our knowledge of the active process grows, transgenesis should also allow tests of more specific hypotheses. Confirming that the active process operates at a Hopf bifurcation, for example, requires the identification and experimental manipulation of the control parameter that poises the system there. Ca^{2+}-dependent channel reclosure is an attractive candidate for the active process, owing to its ability to function at frequencies of tens of kilohertz (Choe et al., 1998), but this process can operate over only the limited range of bundle positions in which Ca^{2+} binding modulates the open probability of the transduction channels. Myosin-based adaptation may provide an adjustment mechanism that maintains the channels within this range (Chan and Hudspeth, 2005a). If active hair-bundle motility is sensitive to protein phosphorylation, as suggested by pharmacological experiments (Martin et al., 2003), site-directed mutagenesis of putative phosphorylation sites will test the contribution of myosin-1c to the active process.

A second promising approach involves in vitro studies of individual myosin molecules or small ensembles of them. It is apparent that myosin-1c—with or without other myosin isoforms—is intimately involved in active hair-bundle motility. Biochemical approaches have recently demonstrated that myosin-1c has several properties that accord with its function in adaptation. In particular, tight binding of this molecule to actin is promoted by mechanical strain (Batters et al., 2004a; Laakso et al., 2008), a feature that explains the capacity of adaptation motors to maintain resting tension in tip links. Moreover, binding is reduced by Ca^{2+} (Ademek et al., 2008), a property hitherto

unknown in other isoforms but quite consistent with the evidence that Ca^{2+} entry through transduction channels promotes the downward slipping of adaptation motors during adaptation to excitatory stimuli. Using the armamentarium of techniques developed for the study of single mechanoenzyme molecules, investigators can now inquire whether myosin-1c has such expected properties as the ability to respond to the rapid Ca^{2+} transients that trigger fast adaptation and that might effect active hair-bundle motility at high frequencies. It should also be possible to ascertain whether the binding of Ca^{2+} to myosin-1c is able to perform mechanical work on an external load, as would be required if the transmembrane Ca^{2+} gradient powers the active process.

The third experimental approach involves the investigation of mammalian cochlear preparations that display the active process in vitro. A fundamental impediment to understanding how the ear's amplifier operates is the dichotomy between two domains of research. On the one hand, studies of intact animals have provided a wealth of data about the active process, and in particular have delineated the four characteristic features discussed earlier. On the other, single-cell research has demonstrated the bases of active hair-bundle motility and of membrane-based electromotility. The challenge lies in connecting the macroscopic with the microscopic observations: precisely how do the two cellular mechanisms account for the four features of the active process? Despite the cochlea's fragility, in vitro preparations can display features of the active process while affording access to hair cells for pharmacological and electrophysiological intervention (Chan and Hudspeth, 2005a, 2005b; Hudspeth and Chan, 2006). If suitable preparations can be made from the murine cochlea, it should be possible to test the effects on the active process of the variety of recently developed transgenic tools for the manipulation of cells by light-activated proteins. Investigations of the active process now occupy a substantial community of researchers, so the outstanding problems in the field are likely to yield in the next few years.

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